EXHIBIT G

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The quest for non-invasive delivery of bioactive macromolecules: A focus on heparins

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Abstract

The development of a non-invasive drug delivery system for unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) has been the elusive goal of several research groups since the initial discovery of this glycosaminogylcan by McLean in 1916. After a brief update on current parenteral formulations of UFH and LMWHs, this review revisits past and current strategies intended to identify alternative routes of administration (e.g. oral, sublingual, rectal, nasal, pulmonary and transdermal). The following strategies have been used to improve the bioavailability of this bioactive macromolecule by various routes: (i) enhancement in cell-membrane permeabilization, (ii) modification of the tight-junctions, (iii) increase in lipophilicity and (iv) protection against acidic pH of the stomach. Regardless of the route of administration, a simplified unifying principle for successful non-invasive macromolecular drug delivery may be: "to reversibly overcome the biological, biophysical and biochemical barriers and to safely and efficiently improve the in vivo spatial and temporal control of the drug in order to achieve a clinically acceptable therapeutic advantage". Future macromolecular drug delivery research should embrace a more systemic approach taking into account recent advances in genomics/proteomics and nanotechnology.

Keywords

Alternative routes; Bioactive macromolecule; Drug delivery; Non-invasive; Heparin

1. Introduction

In recent decades, several promising new anticoagulants have been evaluated. It has been a challenge to determine which of these agents presently under development will provide the greatest efficacy with the greatest degree of safety at a reasonable cost [1,2]. Heparin, a widely accepted and proven anticoagulant discovered by McLean in 1916 [3] has survived more than 80 years clinical experience [4]. This drug is still essentially administered in clinics by injections which present several limitations for effective pharmacotherapy of thrombosis. To overcome these limitations, perhaps a non-invasive and improved heparin delivery system may be needed to enhance patient compliance and minimize adverse effects. For the purpose of this review and for the sake of simplicity, the term heparin hereafter refers to both unfractionated (UFH) and low molecular weight heparins (LMWHs). Whenever applicable, the distinction will be made between UFH and LMWHs. Besides its original therapeutic use as an anti-coagulant, other potential applications of heparin for a vast array of human diseases have been identified [5]. The potentially wide-ranging clinical importance of this bioactive macromolecule warrants the building of better heparin [6] and the development of better

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heparin delivery system. All the foregoing aspects of heparin are beyond the scope of this manuscript which is focused on delivery systems.

Recent reviews related to heparin delivery focused mainly on the oral delivery of heparin [7–9]. To our knowledge, the first attempt of a comprehensive manuscript for alternative routes of heparin delivery dates back to 1964 which covers limited routes of drug administration [10]. The latter review manuscript dealt mainly with UFH probably because information related to LMWH was not available at that time. Since then, it is noteworthy that a lot of efforts have been made by several investigators using newer heparins (e.g. LMWHs), other alternative routes of drug delivery and/or new method of drug delivery. As a contribution to the consolidation of knowledge gathered on heparin delivery to date, this review will focus mainly on the progress and prognostication involving the non-invasive route of heparin delivery. To achieve this goal, we have consulted the US Food and Drug Administration electronic orange book and current literature on heparin containing drug delivery systems (DDS). After a brief commentary on the current route of administration in clinics (the parenteral route), we examine different DDS investigated for each major non-invasive routes including oral, sublingual, rectal, nasal, pulmonary and transdermal routes.

2. Parenteral delivery of heparins

Table 1 shows some examples of UFH and LMWH formulations approved by the US Food and Drug Administration for clinical use since 1982. UFH and LMWHs have traditionally been administered via the parenteral route (intravenous or subcutaneous injection). Newer heparin derivatives such as fondaparinux (a pentasaccharide) and its analogue idraparinux are still administered subcutaneously [11]. Recent strategies to improve parenteral delivery of heparins include stents and antibody targeted approaches. Recently, minimally invasive methods involving the use of heparin in drug eluting stents have emerged [12–15]. Heparin-loaded zein microsphere films have been recently shown to significantly improve the hemocompatibility of drug eluting stents for cardiovascular applications [16]. Non-eluting stents have clinically reduced thrombotic complications following stent implantation [17]. The first compounds considered for stent-based delivery, such as heparin, were chosen on the basis of promising tissue culture and animal experiments, and yet they have failed to stop restenosis clinically. The application of continuum pharmacokinetics to examine the effects of transport forces and device geometry on the distribution of stent-delivered hydrophilic and hydrophobic drugs showed that mere proximity of delivery devices to tissues does not ensure adequate targeting, because physiological transport forces cause local concentrations to deviate significantly from mean concentrations [18]. It is important to note that stent performance is also influenced profoundly by stent design and configuration. A stent-less local delivery system for anti-restenotic agents based on antibodies targeted to cross-linked fibrin was successful in the targeted delivery of UFH and LMWH to injured areas of the artery wall without systemic complications, suggesting that the local delivery of such agents may minimize systemic effects and bleeding complications [19]. The antibody targeted triggered, electrically modified prodrug-type strategy (ATTEMPTS) also used a similar approach [20].

Though invasive or parenteral formulations are available for heparin delivery, they are poorly accepted by patients and present several restrictions in terms of manufacture (they should be pyrogen and particulate free, isotonic, sterile, and stable) and in terms of pharmacokinetic and pharmacodynamic aspects that may be overcome by non-invasive delivery strategies.

3. Obstacles for non-invasive delivery of heparins

Currently, there is a clinical need for a non-invasive anticoagulant to replace warfarin for long-term prophylaxis and treatment of patients with venous and arterial thrombosis [1]. Improved

delivery systems for heparins are attractive solutions to achieve this goal for numerous reasons as follows: heparins are the anticoagulant of choice in pregnancy as they do not cross the placenta and administration during pregnancy is not associated with undesirable effects in the fetus or neonate. Prevention of thromboembolism in patients with atrial fibrillation and prosthetic valves are still areas where there is a need for new anticoagulant drugs [1]. LMWHs can be used safely and effectively to treat outpatients with proximal deep-vein thrombosis [21]. The design of an ideal DDS for heparins that could circumvent current pharmacokinetics, biophysical and antihemostatic limitations will have tremendous benefits including improvement of patience compliance due to avoidance of pain during injection adding convenience, safety and efficacy to thrombosis therapy. Such ideal DDS for heparins may therefore reduce the healthcare cost because thrombosis is the discharge diagnosis of more than a quarter-million patients in U.S. hospitals annually [21].

The lack of non-invasive delivery options for heparins may result in limited clinical use and poor patient compliance. The major barriers hindering the delivery via the non-invasive route for heparin include: (i) enzymatic degradation due to heparinase present in the liver [22] and the intestinal microflora related to *Bacteroides* spp. [23,24], (ii) chemical instability at acidic pH of the stomach [25] and (iii) limited absorption through the epithelial/mucosal barrier. It has been shown that desulfation of heparin and the metabolism of the glycoside residue may occur in the acidic pH of the stomach unless heparinase derived heparin fragment is used [26]. Selective *N*-deacetylation and *N*-desulfation of the glucosamine residues of heparin have been shown to affect both its anticoagulant activity and in vivo disposition characteristics [27]. The poor absorption of heparins across the physiological barrier is due to their hydrophilic nature, negative charge, and relatively large molecular weight. The absorption of large hydrophilic macromolecules such as heparin may be limited to the paracellular pathway, which consists of aqueous pores created by the cellular tight junctions [28]. For a drug with a molecular mass beyond 500–700 Da, the bioavailability decreases with an increase in the molecular mass [28]. Even if absorbed through mucosal barriers, another obstacle to efficient heparin delivery by non-invasive route is its susceptibility to hepatic metabolism by heparinase and the preferential concentration of heparin in the endothelium [29,30].

4. Heparin containing DDS for sublingual route

The oral mucosa, floor of mouth, underside of tongue and gingival mucosa offers excellent accessibility, is not easily traumatized and avoids degradation of macromolecular drug resulting from oral gastrointestinal absorption and first-pass hepatic metabolism [31]. The early claims for sublingual delivery of heparins [32–36] have been challenged and did not survive critical investigations [10,37,38]. For example, tablets containing 20,000 U of UFH with and without ethylenediamine tetraacetic acid (EDTA) as penetration enhancers did not show any significant changes in bioactivity in the plasma of treated patients [10]. The inconsistency of earlier data may reflect the difference in the properties of heparin preparation, the sensitivity and the accuracy of the bioactivity assessment method that was based on optical density at that time [39]. Nevertheless, it is noteworthy that, although promising, the sublingual route of heparin administration has not been extensively investigated for LMWH. This may probably be due to the fact that heparins would have to permeate approximately 30–40 cell lines until they reach the first blood vessels in the lamina propria. Though the successful development of buccal heparin delivery systems seems unlikely based on the above fact, with the evolving concept of building better heparin, this route deserves further investigation in the quest for non-invasive delivery methods for heparin.

5. Heparin containing DDS for oral route

By far the most convenient and preferred route of drug administration is the oral route. Therefore, the oral delivery of heparin is one of the most intensively studied delivery strategies [7]. Successful clinical use via the oral route has been hindered due to the obstacles described earlier. Numerous attempts have been made to develop an oral delivery system for heparins both in vitro and in vivo. In vivo studies involved different animal models such as mice [40, 41], rat [42–55], rabbit [56–58], dog [59], pig [53], primates [48] and human [60–65]. Table 2 underlines examples of oral formulation tested in humans.

Overall, a variety of formulation strategies have been investigated to circumvent the current obstacle to oral delivery of heparins previously underlined. These strategies may be classified based on the following mechanism.

a. Cell-membrane permeabilization using bile salts and derivatives [66–68] and polycationic lipophilic-core dendrons (partial dendrimers) [69].

(ii) Tight-junction modifications using absorption enhancers such as labrasol [70], sulfonated surfactants [71], EDTA acid [44], saponins [72], chitosan derivatives [73], thiolated polycarbophil system [42,52,74], carbopol 934P [73], sodium caprate [55], and Zonula occludens toxin synthetic peptide derivative AT1002 [75].

(iii) Increasing the drug lipophilicity: covalent attachment to lipophilic molecules such as dimethyl sulfoxide and deoxycholic acid conjugates [66–68], the use of carriers such as organic acids [76], sodium *N*-[10-(2-hydroxybenzoyl) amino] decanoate [77] (SNAD), sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC) [8,65,78], diamine salt (ITF 1331 or counterion no. 4 [56], microemulsion formulations [66,79], polyion complex micelles [80], liposomes [59] and dendrons [69]. Microemulsions are potential drug carrier systems for oral, topical, and parenteral administration [81]. These typically consist of water, oil, and amphiphilic compounds (surfactant and co-surfactant) which yield a transparent, single optically isotropic, and thermodynamically stable liquid. The main difference between macroemulsions and microemulsions lies in the size of the particles of the dispersed phase: these are at least an order of magnitude smaller in the case of microemulsions (10–200 nm) than those of conventional emulsions (1–20 μm). Drug penetration enhancement from microemulsions is mainly due to an increase in drug concentration which provides a large concentration gradient from the vehicle to the physiological barrier. Furthermore, it has been suggested that the surfactants and the oil from the microemulsion interact with the rigid lipid bilayer structure and acts as a chemical enhancer. The new area of polymer therapeutics [82] includes polymeric micelles containing covalently bound drugs such as heparin. Liposomes [83] are microscopic aggregates of highly ordered lipid molecules which are normally dispersed in a hydrophilic solvent, typically water.

(iv) Protection against acidic pH of the stomach: enteric coating [84,85], use of alginate/chitosan/PEG microparticles [86] and polymeric nanoparticles [57]. Results obtained by this strategy are controversial. For example, the complexation of one fraction with glycine (to adjust the ionization of the drug), and the use of gastroresistant capsules administered directly into the stomach did not result in significantly increased absorption, although large doses were administered (15,000 anti-Xa U/kg) [84]. However, improvement of heparin absorption from the gastrointestinal tract was claimed by a combination of suppression of ionization and selection of molecular size [87] and after enteric-coating [85]. Microparticles [88] may be obtained by microencapsulation, a technology devoted to entrapping solids, liquids or gases inside one or more polymeric coatings. Similar technologies are used

to produce nanoparticles. The main difference between nano- and microparticles is their size, the former are typically less than 1 μm while the latter are typically above 1 μm. For successful heparin delivery using carriers, both the drug and biological characteristics of the carrier, carrier–gut interactions, the dynamic nature of such interactions, the varied modes of uptake in vitro and in vivo, and the concerns of targeting to the gut epithelium to encourage more efficient uptake of nanoparticles need to be elucidated [89]. For example, the differences in the uptake of the mucoadhesive polysaccharide chitosan (CS)-coated systems (solid lipid core or oily core) by the Caco-2 cells did not have a consequence in the in vivo behaviour [90] indicating the difficulty of obtaining appropriate in vivo and in vitro correlation with these novel DDS. Whether or not these strategies can be utilized for the routine administration of heparin from the gut remains to be known.

Due to the large amount of preliminary data on the oral delivery of heparins (in human and animal models), we have summarized the data obtained in clinical trials in Table 2. Overall, novel and reversible absorption promoters show promise for the oral delivery of heparin. It appears that effective and safe delivery of heparins by the oral route would be clinically relevant not only for thrombosis but also for other localized disease conditions such as gastric ulcer [50].

6. Heparin containing DDS for rectal route

The rectal administration of drugs has been extensively reviewed [91]. Several strategies have been investigated for the rectal delivery of heparin. Table 3 summarizes the examples of in vivo studies involving rectal absorption of heparins in the animal model. The main formulation strategies implemented focused on modification of cell membrane permeability using sodium cholate [92], bile salts [93] and sodium lauryl sarconsinate [94]. The oil emulsion improved the bioavailability of glycosaminoglycan sulfates at least 20 times [94]. Rectal absorption of heparin in rabbits in the presence of non-surfactant adjuvants [95] has also been investigated. These studies showed that the alteration or disruption of tight junctions plays an important role in the absorption of heparin. Unfortunately, limited data are available on the toxicity and in vivo performance via this route.

In order to gain a therapeutic response after rectal administration, heparins have to permeate the absorption membrane based on the mucus layer and the epithelial tissue in significant quantities. The transport of heparin across the rectal membrane may be further improved by the co-administration of mucolytic agents and permeation enhancers. In addition, a combination of oral and rectal formulations may succeed when one route, alone, is not successful such as in the case of inflammatory bowel disease [96]. The effective and safe delivery of heparins by another body orifice namely the vagina may be clinically relevant not only for systemic thrombosis but also for the improved pharmacotherapy of other localized disease conditions and for patients who have undergone gynecological surgery [97], patients with septic pelvic thrombophlebitis (a major complication of endometritis) [98] and in cases of postpartum ovarian vein thrombosis after vaginal delivery [99].

7. Heparin containing DDS for nasal route

In recent years, the nasal route has received a great deal of attention as a convenient and reliable method for the systemic administration of drugs. Although this route is currently being used in the clinics for the systemic administration of several drugs, it is a recently emerging area [100].

Several strategies have been investigated for the intranasal delivery of heparin. Table 4 summarizes the main strategies used via this route. Investigations have been conducted in rat

[101,102] and human nose [103–105]. Delivery systems used include solution vapor and nebulizers as shown in Table 4. The major barriers to nasal drug absorption are enzymatic degradation, the inability of the nasal mucosa to enable transport of any molecule larger than 1 kDa, and the relatively short residence time of substances in the nose. In the case of heparin delivery, recent efforts to overcome these problems use penetration enhancers such as alkylglycosides [101,106]. The mechanisms, by which these enhancers increase absorption, remain to be elucidated. It is generally accepted that the absorption enhancers promote absorption by a direct effect on the membrane. The human nose can accommodate a dose of 25–200 μl per nostril. Based on heparin solubility and therapeutic dose requirement, a relatively higher volume may be needed leading to potential drainage out of the nose. This limitation of dose volume should be taken into account while formulating a nasal drug delivery system for heparin. Absorption via the nasal route may also be affected by the site of administration of formulation in the nose. The anterior part of the nose provides greater contact between nose and drug whereas a formulation applied to the posterior part of the nose is removed rapidly by the mucociliary clearance mechanism of the nose [107].

A successful nasal heparin delivery system would offer numerous advantages including rapid onset of action and avoidance of hepatic first-pass metabolism. An improved understanding of the structure and function of tight junctions in the nasal epithelial barrier is needed before significant improvements in the delivery of large molecules such as heparin can be made. The effective and safe delivery of heparins via this route may be clinically relevant not only for systemic thrombosis but also for other localized disease conditions (e.g. allergies [103,104]).

8. Heparin containing DDS for pulmonary route

The pulmonary route of drug delivery is well established in the treatment of lung diseases such as asthma. In recent years, this technology has progressed to the extent that it is now possible to deliver macromolecules to the systemic circulation via inhalation. Bioengineered particles may be created in liquid form from devices specifically designed to create an unusually fine size distribution or solid particles that possess a mixture of drug and excipient, with defined shape, size, porosity, and drug release characteristics [108].

Several strategies have been investigated for the pulmonary delivery of heparins. The absorption of LMWH from the respiratory tract is hampered due to excessive hydrophilicity and surface charges. The delivery of heparin via this route would be especially beneficial, in the case of PE owing to the prospect of targeted delivery at the site of action. Table 5 shows various strategies that have been employed to increase drug absorption via the pulmonary route. Animal models tested by this route include mice [109], rat [110,111], guinea pig [112], rabbit [113,114], sheep [115] and dog [109]. Very few investigations have been performed in humans as shown in Table 5.

One of the challenges in pulmonary drug delivery is the reproducible placement of drug at the site of absorption in the alveoli. This issue has received considerable attention, and resulted in the design and development of varied devices to provide consistent drug delivery to the deep lung tissue [116]. The effective and safe delivery of heparins by this route may be clinically relevant not only for the systemic effect against thrombosis but also for the improvement of localized pharmacotherapy such as in cases of allergy and asthma management [117–119].

9. Heparin containing DDS for transdermal route

The skin provides an attractive and readily accessible site for drug delivery. The transdermal delivery of heparin is of interest, because drug absorption across the skin avoids first-pass metabolism. Previous reviews [120,121] provide an insight into the in vitro and in vivo studies on percutaneous absorption of heparins. Recently, various novel strategies (Table 6) have been

investigated to enhance the transdermal delivery of heparin. This route offers several advantages over traditional drug delivery systems. These include minimization of pain and the prospect of sustained drug release. The major disadvantage to transdermal heparin delivery is the low bioavailability of bioactive macromolecules through the skin mainly due to the presence of the stratum corneum. Strategies that have been used to overcome this barrier and increase the permeability of the skin to heparin include penetration enhancers [122,121], liposomes [123,124], phonophoresis [125,126], electroporation [127–129], iontophoresis [126,130], needle-less injections [131] and microfabricated microneedles [132]. Several excipients are able to promote the transport of an active substance across the skin barrier by a variety of mechanisms including extraction of lipids from the stratum corneum, alteration of the vehicle/skin partitioning coefficient, disruption of the lipid bilayer structure, displacement of bound water, loosening of the horny cells and delamination of the stratum corneum. Liposomes have been previously defined elsewhere in this manuscript. Phonophoresis (or sonophoresis) uses ultrasound energy [133] in order to enhance the skin penetration of the drug. When the skin is exposed to ultrasound, the waves propagate to a certain level and cause several effects (cavitation and energy loss) that assist skin penetration. The force of cavitation leads to the formation of holes in the corneocytes, enlargement of intercellular spaces, and perturbation of stratum corneum lipids. The energy loss results in a rise in the temperature which increases the fluidity of the stratum corneum lipids and directly increases the diffusivity of molecules through the skin barrier. In contrast to iontophoresis where a low voltage is applied, electroporation [127–129] requires a large voltage treatment for a short period (10 μs to 100 ms) to produce transient aqueous pathways across the skin barrier. These pores allow the passage of macromolecules via a combination of diffusion, electrophoresis and electroosmosis. Using tools from the microelectronics industry, microneedles [132] have been fabricated with a range of sizes (10 to 200 μm in height and 10 to 50 μm in width), shapes (solid or hollow) and materials (biodegradable or not). Microneedle arrays connected to a reservoir are applied to the skin surface such that they pierce the upper epidermis far enough to increase skin permeability and allow drug delivery, but too short to cause any pain to the receptors in the dermis. Therefore, in this case there are no limitations concerning polarity and molecular weight of the delivered molecules. The needle-less system (namely J-Tip®) used for heparin delivery is a sterile, single use, disposable device that contains its own source of propellant consisting of liquid CO_2. It is important to point out that the needleless injections and microfabricated needles may be construed or viewed as invasive parenteral routes. However, we have included these systems under transdermal delivery systems because they are often emerging painless alternative physical methods intended to systemically deliver drugs through the skin beside the above chemical, electrical and ultrasound based methods.

One major drawback in the case of drug delivery via the transdermal route is the potential local irritation at the site of absorption. In spite of this, evidence to support preferential binding of heparin to keratinocytes and its high transcutaneous permeation through the skin suggests that it may be an excellent candidate for use in the transdermal delivery of other drugs. Another additional advantage in the delivery of heparin by this route may be an improvement in the treatment of superficial venous thrombosis [123].

10. Perspectives on the current challenges of bioactive macromolecule/ heparin delivery

The major challenges which need to be overcome for effective and safe delivery of heparins are instability in the organism (e.g. related to heparinase or at low pH), low permeability through the biological tissue and better spatial and temporal control over the pharmacokinetics and pharmacodynamic properties. The various non-invasive routes of delivery of LMWH show promise for patient compliance in thrombosis management but none of them is yet to be proven safe and effective for clinical use. Future advances in this field may be based on: (i) a better

understanding of the microbiota of the site of administration and their influence on the bioactive macromolecules, (ii) our ability to smartly mimic bacterial invasion process, (iii) the use of newer methods in genomics and in nanotechnology, (iv) development of in silico predictive model for bioavailability based on physicochemical properties to decrease the probability of failure and (v) a better control of the production cost for routine use and affordability by diverse human population. For example, the distal human intestine represents an anaerobic bioreactor programmed with an enormous population of bacteria including *Bacteroides* sp. that secrete heparinase. This microbiota and its collective genomes (microbiome) provide us with genetic and metabolic attributes [134] that may differently affect the fate and stability of different bioactive macromolecules. Moreover, invasive bacteria actively induce their own uptake by phagocytosis in normally nonphagocytic cells and then either establish a protected niche within which they survive and replicate, or disseminate from cell to cell by means of an actin-based motility process [135]. An ideal noninvasive system should mimic these natural invasion processes without inducing any adverse effect. In term of technologies, recent advances include phage display. The latter is a simple functional genomic methodology for screening and identifying protein–ligand interactions and is widely used in epitope mapping, antibody engineering and screening for receptor agonists or antagonists [136]. For example this technique has been used to identify AT1002, a hexapeptide derived from Cholera Zonula occludens toxin derivative that is a promising penetration enhancer for macromolecules including heparin [75]. Therefore, one may reasonably speculate that some methods derived from current advances in genomics/proteomics may be useful to address the current challenge of macromolecular drug delivery. The use of some relatively newer nanotechnological methods/tools [137] such as dynamic force spectroscopy [138], microfluidics [139] and ion trap tandem mass spectrometry [140] to delineate underlying physicochemical mechanisms and probe the interaction at the interface between biology and physico-chemistry may lead and to successful non invasive delivery of these drugs. Another challenge to be addressed with these macromolecules is the development of predictive model for their bioavailabilities based on their complex molecular properties and perhaps conformational properties in search of optimization process. Such efforts have been successfully developed for small chemical entities either for oral [141,142] or transdermal [143] routes but there is a knowledge gap for therapeutic macromolecules. Additional biological and clinical studies are also required for these novel delivery systems in order to confirm their safety and efficacy after a more systematic in silico and in vitro study to decrease the probability of failure. The cost for routine use of such novel DDS may be often prohibitive and should also be minimized to justify their choice over conventional drug delivery methods.

11. Conclusions

The various non-invasive routes of delivery of LMWH show promise for patient compliance in thrombosis management but none of them is yet to be proven safe and effective for clinical use. It is noteworthy that one limitation of this review is that bioavailabilities data could not be critically analyzed and compared. This limitation is due to the large differences between operating procedures. For example, there was a wide variety between the nature/type of heparin used in each study, the various doses administered, the differences in the animal species used, method of administration, blood sample analysis methods, and in the data collection and treatment.

Future advances in this field may be based on: (i) a better understanding of the microbiota of site of administration and their influence on the bioactive macromolecules, (ii) our ability to smartly mimic bacterial invasion process, (iii) the use newer methods in genomics (e.g. phase display) and in nanotechnology, (iv) development of in silico predictive guidance for bioavailability based on physicochemical properties to decrease the probability of failure and (v) a better control of the cost for routine use of such novel DDS to justify the choice over

conventional delivery systems. Future drug delivery research on bioactive macromolecules such as heparin should embrace a more systemic approach taking into account data not only from physico-chemistry, pharmacokinetics/pharmacodynamics of the drug but also knowledge gained from advances in genomics/proteomics and nanotechnology.

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Table 1

Examples of parenteral formulations of heparins and derivatives approved by US Food and Drug Administration

Approval date (dosage strength)	Type of heparin (proprietary name)	Developer/marketer	Delivery method
January 30/93 (10,000 IU/mL)	LMWH: dalteparin (Fragmin)	Pfizer	Injection
March 29/93 (30 mg/0.3 mL)	LMWH: enoxaparin (Lovenox)	Aventis	Injection
July 14/00 (20,000 IU/mL)	LMWH: tinzaparin (Innohep)	Pharmion	Injection
Prior January 1/82 (1000; 10,000; 20,000 U/mL)	UFH: heparin sodium	Am. Pharm Partners	Injection
July 20/92 (10,000 U in dextrose 5% in 100 mL)	UFH: heparin sodium	B. Braun	Injection
Prior January 1/82 (1000; 5000, 10,000 U/mL)	UFH: heparin sodium	Baxter Health Care	Injection
February 28/95 (10; 100 U/mL)	UFH: heparin sodium	Hospira	Heparin
January 1/82 (1000; 5000; 10,000 U/mL)	UFH: heparin sodium	Pharmacia and Upjohn	Heparin
October 10/95 (1000 U/mL)	UFH: heparin sodium	Marsam Pharms LLC	Injection
Dec 7/01 (2.5 mg/0.5 mL)	Pentasaccharide: fondaparinux sodium (Arixtra)	Glaxo Smith Kline	Injection

LMWH=low molecular weight heparin and UFH=unfractionated heparin.

Table 2

Examples of clinical trials on oral absorption of heparins

Type of heparin	Drug delivery system	Mechanism of action	Results	Ref.
UFH	Piece of bread (7 g)	Effect on lipid metabolism	Slight increase in aPTT, marked decrease in triglyceride, slight decrease in cholesterol, HDL and LDL	[61]
UFH	In 0.9% saline	Binding to cytokine and potent immune modulatory action	Clinical improvement of rheumatoid arthritis	[62]
LMWH	Buffer pH 4.0 and 7.0	NA	No detectable plasma activity after oral administration of either LMWH delivery system	[60]
LMWH	NA	Anticoagulant and antithrombotic properties	Treatment of corticosteroid-resistant ulcerative colitis in combination with sulfasalazine	[63]
UFH	SNAC in 10/15 mL syrup	Mediation of passive absorption of non-covalent complex	Oral heparin/SNAC can be safely delivered to the postoperative THA patient	[65]
UFH	Liquid in 200 mL of water	Diffusion? Not yet elucidated	Plasma anti-Xa activity increased as soon as 5 min after drug administration, peaked at 120 min, and was still increased 72 h after administration	[64]

Bid: twice daily, tid: three times daily, NA: not available. PE: penetration/absorption enhancer. SNAC: sodium *N*-[8-2-hydroxybenzoyl)amino]caprylate. THA: total hip arthroplasty. F=absolute bioavailability.

Table 3

Examples of in vivo studies on rectal absorption of heparins

Animal model	Type of heparin	Drug delivery system	Mechanism of action	Results	Ref.
Rat	UFH	Oil+sodium laurylsarcosinate (1–3 mg/kg)	Penetration enhancing effect of surfactant	Dose-dependent effects and kinetics comparable to those of IM administration. F improved by 20× at least	[94]
Rodent Primates	[35S]heparin	Sodium cholate (Sch) or sodium deoxycholate (DOC)	Penetration enhancing effect of Sch. Abd DOC	Absorption through the rectal mucosa with DOC only. Partial thromboplastin time (PTT) was less sensitive test of heparin absorption than the plasma lipase activity	[93]
Rat Human	LMWH (Fragmin)	Microenema Sch (10–20 mg/mL)	Penetration enhancing effect of surfactant	Sch facilitates absorption of LMWH	[92]

LMWH=low molecular weight heparin and UFH=unfractionated heparin.

Table 4

Examples of in vivo studies on nasal absorption of heparins

Animal model	Type of heparin	Drug delivery system	Mechanism of action	Results	Ref.
Rat	Enoxaparin, dalteparin, UFH	0.25% Tetradecylmaltoside (TDM) solution	Increased nasal permeability	Increased bioavailability of LMWH from ~4% in the absence of TDM to ~19% in the presence of TDM but not with UFH	[106]
Human	UFH	Solution vapor	Anticoagulant action and vagal activation	Prolonged onset and completion of blood clotting and decreased heart rate	[105]
Human	UFH	Nebula nebulizer	Possible neutralization of eosinophil cationic protein and reduction of eosinophil recruitment	Protection with respect to nasal allergen challenge	[104]
Human	UFH	Nebulizer connected to a nasal adaptor	Protective role against AMP provocation by inhibition of mast cell activation	Significant attenuation of the release of histamine and tryptase induced by AMP challenge	[103]

UFH=unfractionated heparin and AMP=2-amino-2-methyl-1-propanol or β-aminoisobutyl alcohol.

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Table 5

Examples of in vivo studies on pulmonary absorption of heparins

Animal model	Type of heparin	Drug delivery system	Mechanism of action	Results	Ref.
Newborn rat	UFH	Serpin-heparinoid complex	Effect on free thrombin generation	Inhibitors of thrombin generation on fetal distal lung epithelium superior to the corresponding non-covalent mixtures	[110]
Rat	Enoxaparin, dalteparin, UFH	Tetradecyl-beta-maltoside (dimethyl-beta-cyclodextrin	Increased drug transport by both agent acting mainly on cell membrane	Enhanced pulmonary absorption of LMWH	[111]
Rat	UFH	Krebs–Ringer phosphate solution	Simple diffusion	50% absorption occurred in 9.2 h. No saturation of absorption when conc. was raised to 1000×	[144]
Guinea pigs	UFH= multiparin, LMWH=Fragmin	Chlorocresol+in nebula nebulizer	Anti-inflammatory properties	Significant inhibition of allergen-induced eosinophil infiltration when administered directly to the airways	[112]
Guinea pigs	UFH, LMWH	Ultrasonic nebulizer	Possible inhibition of inflammatory cells and reduction of inflammatory mediator release	Anti-airway anti-allergic inflammatory activity	[117]
Rabbit	UFH	Ultrasonic nebulization+tight fitting mask	Effect on alveolar fibrin generation	Suppression of soluble collagen and hydroxyproline accumulation, and abrogation of histologic features of lung fibrosis	[113]
Rat Rabbit	UFH, LMWH (ardeparin)	Solutions and powder of micro particles in insufflator	Influence on tight junction complex of epithelial cells	Rapid onset of action ($t_{1/2}$) =40 min. Inhibition of thrombosis and emphysema	[114]
Sheep	UFH	Disposable raindrop nebulizer	Blockade of inositol triphosphate receptors in various tissues	No effect on baseline specific lung resistance but attenuated antigen-induced bronchoconstriction in a dose-dependent fashion	[115]
Mice	UFH		Anticoagulant properties	No evidence of acute or long-term toxicity in lung or other tissues. Duration of response increased with dosage	[109]
Rat Dog Human		Ultrasonic nebulizer			
Human	UFH	Disposable raindrop nebulizer	Non-anticoagulant action more likely related to a modulation of mediator release	Inhaled heparin prevents exercise-induced asthma without affecting histamine-induced bronchoconstriction and aPTT	[145]
Human	UFH	Sidestream jet nebulizers	Anticoagulant	No effect on pulmonary function	[146]

Animal model	Type of heparin	Drug delivery system	Mechanism of action	Results	Ref.
				Dose-dependent increased of anti-Xa activity	

Table 6

Examples of studies on transdermal absorption of heparins

Experimental model	Type of heparin	Drug delivery system	Mechanism of action	Results	Ref.
Human epidermis in vitro	UFH	Short, high-voltage (HV, U~100 V) pulses	Alter ionic and molecular transport	First demonstration of a chemical enhancer effect for transdermal transport by HV pulsing	[129]
Rat skin in vitro	UFH	Electroporation device (voltage across skin ~40 V=iontophoresis	Stabilize skin permeability caused by high voltage pulse	Skin electroporation increased transdermal mannitol up to 5× with macromolecules	[128]
Rabbit ear in vitro	LMWH (Certoparin), UFH	Solution	Local anticoagulant action	Topical administration of LMWH prevents the occurrence of thrombosis at the traumatic anastomosis site to a similar degree as heparin	[122]
Pig skin in vitro	^{3}H-heparin	Sonicator (frequency: 20 kHz), iontophoresis with Ag/AgCl disc electrode (0.45 mA/cm^{2})	Formation of transport pathways by Ultrasound+Control of flux by electric current	Ultrasound under low frequency (20 kHz) with iontophoresis enhances transdermal transport of heparin	[126]
Human skin in vivo	LMWH: dalteparin sodium	J-Tip® needle-less injection device	Mechanical removal/bypass of the stratum corneum	LMWH equally effective as the standard needle but was significantly more comfortable. Ease of administration	[131]
Human cadaver skin in vitro	UFH LMWH	Liposome Phospholipon® 80 and sphingomyelin	Penetration enhancer effect	Molecular weight (LMWH>UFH) and formulations influenced the penetration. No conclusion of pharmacological effect	[124]
Pig skin in vitro	LMWH (dalteparin)	Low frequency ultrasound (20 kHz)	Ultrasound mediated-increased skin permeability	Sustained anti-factor Xa (aXa) levels in the blood (F=6% in 24 h). SC and IV resulted in temporary elevations of aXa	[125]
Rat skin in vivo	UFH				
Human skin in vivo	UFH	Liposome spraygel (Lipohep® 2400 IU/g)	Antithrombotic and antiphlogistic action	Efficient with compression therapy in the treatment of superficial vein thrombosis	[123]